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THERAPEUTIC POTENTIAL OF *PEGNUM HARMELA* AGAINST *SCHISTOSOMA BOVIS* IN BUFFALOES

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Abstract

Background: *Peganum harmala* have many biological and pharmacological activities such as antifungal, antibacterial, analgesic and anti-inflammatory. The present study was carried out to evaluate the antischistosomal activities of *Peganum harmala* with special reference to bovine schistosomiasis in naturally infected buffaloes.
Methods and Materials: A total of 54 naturally infected buffaloes with "Schistosomiasis" of ages around five years were used for anthelmintic studies. All of the buffaloes were properly marked so as to make a distinction from one other. A doctor of veterinary was available for daily checkup. Samples prepared according to the available methods in literature.
Results: In the present study, naturally infected buffaloes with *Schistosoma bovis* were treated with *Peganum harmala* (Harmal, seed), with dose 75 mg/kg, 150 mg/kg and 225 mg/kg body weight respectively, and their impact on various parameters like eggs reduction, milk production, weight gain and feed intake was noted. Furthermore, their efficiency (%) was evaluated with "Praziquantel" (allopathic drug) at "10mg/Kg body weight" dose level. After first dose of "Praziquantel" hundred percent effectiveness was noted, while the same result obtained for herbal drug after giving second dose of "225mg/kg body weight". To evaluate the effect of herbal medicines on protein portions, sera of treated and control buffaloes were also investigated.
Conclusion: From present findings, it is concluded that the herb *Peganum harmala* can easily replace Praziquantal (PZQ) with almost same efficiency (%). Furthermore, the herb is easily available at cheap price at local market and it will be good for the economy of Pakistan

Key words: *Pegnum harmela, Schistosoma,* infection

Introduction

Plants not only provide food and shelter but also play a friendly role to mankind by preventing and curing different diseases (Charis, 2000). Cattle farmers and pastoralists in various developing countries of the world have long relied on and are still using medicinal plants for treatment of cattle diseases (Lans and Brown 1998). Ethno-veterinary medicines, the medicines that are used by livestock keepers, other than modern synthetic drugs (Mathius and McCorkle 1989), have been reported to be widespread among village livestock producers and herdsmen (Alawa et al 2002). *Peganum harmala* have many biological and pharmacological activities such as antifungal, antibacterial, and MAO inhibition (Diba et al 2011, Abdel-Fattah et al 1997), analgesic and anti-inflammatory (Farouk et al 2008), Antileishmanial activity (Rahimi-Moghaddam et al 2011). *Peganum harmala* is also effective against the ovine malignant theileriosis, highly fatal, acute or sub-acute disease of sheep caused by the tick-borne protozoan parasite. The present study was carried out to evaluate the antischistosomal activities of *Peganum harmala* with special reference to bovine schistosomiasis, parasitic diseases, caused by *Schistosoma bovis* and which is a major problem in many parts of the world (Derakhshanfar et al 2008, Aradaib et al 1995).

Serum protein profile is a very helpful tool to search out an indication on articulated proteins fractions, and to detect alteration in concentration as well as iso type division of the recognized proteins. Furthermore, a sufficient familiarity in the alteration of protein pattern may lead to profound approach into the metabolic growth of several pathologic surroundings and possibly will serve as biomarkers for additional exploration (Miller et al 2009). Key investigative information for the findings of diseases is given by protein biomarkers, like risk of disease progression, and a patient's likely response to drug therapy (Van-der et al 2003). Identification and quantification of individual protein fractions and determination of the normal serum protein profile in a species enables the identification of individuals with changed patterns (Alberghina et al 2011). *Schistosoma bovis* is responsible for "Bovine schistosomosis", which caused permanent grave problems in animals all over the world (Aradaib et al 1995). Thus, it is necessary to analyze the serum protein profile of buffaloes naturally infected with *Schistosoma bovis*, before and after herbal treatment.

Method and Materials

During the present study, a total of 54 infected (with schistosomiasis) buffaloes "5.0±1.15 years" of ages were selected for anthelmintic activity. The selected animals were marked so as to separate them from one another.

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Preparation of Herbal Drugs

*Pegnum harmela (*Harmal) was tried & its efficacy was compared with "Praziquantel". This herbal medicinal seeds were bought from the market and made free from dust by washing and then grinded finely. The medicinal powdered were administrated orally in capsules by using protocol of ("Jhangir*et al* 2003").

Experimental Design

During the trail, the buffaloes were randomly placed in three main groups "A, B and C". Buffaloes in group A were further sub grouped into "A1, A2 and A3", each was comprised of nine buffaloes & these buffaloes were treated with *Pegnum harmela (*Harmal) at dosage rate of "75, 150, 225 mg per kg body weight". Buffaloes included in group B were administrated with "10mg/kg of Praziquantal (PZQ)". Group C buffaloes were kept untreated "control infected". Nine uninfected animals of group D were controlled as normal for the comparison to compare with groups of infected animals. "EPG" were observed by "Mc Master Egg counting technique (Soulsby, 1982)". Animals were surveyed after treatment at "zero, 3rd, 7th and 18th day". Positive animals at 18th day were treated with second dose. After that, on the 21st and 28th days and their faecal samples were investigated. Drug's efficacy was calculated as described by ("Soulsby 1982").

Efficacy (%): "Total no of eggs before treatment" – "Total no of eggs after treatment" X 100
"Total no of eggs before treatment"

"Protein Electrophoresis DS-PAGE"

Using "Sodium deodocylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique, protein fractioning of pre-treatment, post-treatment and control blood sera were analyzed following the method of ("Laemmeli 1970").

Image Capture and Quantification for Protein Fractions

For identification and quantification, the gels were scanned and electrophoretically resolved for protein fractions by "Gene Genius and Image J Gel Documentation Systems".

Result and Discussion

Comparative Efficacy (%) of Peganumharmala(Harmal,) and PZQ

Drug'sefficacy (%) was counted by finding the reduction of eggs in fecal samples after treatment (Table 2). To investigate the difference of control and treated groups, mean percentage of the fluke's eggs in fecal samples were also compared (Table 1).

Efficacy (Group-A) *of Peganumharmala*

At 75 mg/kg b. w. ,on 1st& 2nd dose level of *Peganumharmala* seeds was noted "16.36% and 64.20%" respectively, while high dose rate of 150 mg and 225 mg/kg b. w showed "65.70%, 100% and 96.67%, 100%" efficacy respectively (Table- 2).

Statistical analysis demonstrated considerable decrease ($P<0.05$) in egg output at all dose levels of *P.harmala* and "EPG" also recorded zero in sub-groups "A_2 and A_3" after treatment (Table -1).

Efficacy of Praziquantel (Group B)

100 % effectiveness of PZQ drug at 10mg/kg body weight was noted. Significant ($P<0.001$) decrease in EPG was found which became zero on 18th day after treatment "Table-1".

EPG count was found significantly ($P<0.01$) greater than before (35.0%) in "control group C". Significant ($P<0.01$) difference in PZQ effectiveness was found in comparison, with lowest "75mg/kg dose level" whereas no significant difference was found at "150 mg/kg and 225 mg/kg" dose levels at the end of treatment (Table-2).

Effect on Milk Production

In sub-group A_1, A_2 and A_3, milk production was noted as "2.73±0.3, 2.86±0.2 and 2.53±0.1" liters before treatment while milk production was improved up to "3.0±0.2, 5.26±0.2 and 5.40±0.2 liters", after treatment respectively. Noteworthy increase value ($P<0.01$) was observed in sub group A_2 (85.7%) and A_3 (116%), (Table-3). In group B, high significant value ($P<0.001$) was recorded (126%) in milk increase while untreated control animals (Group C) showed 53.1% significant ($P<0.01$) reduction in milk production (Table-3).

Effect on Body Weight and Feeding

Except animals of group C (non-treated), all other animals showed decrease in body weight non-significantly. 30-96% increased feed intake was noted in all treated animals ($P<0.001$ highly significant). Non-treated group C showed (32.4%) decrease while in animals in Group D (normal group) showed slight increase (2.1%) in feed intake (Table-3).

Serum Protein Profile

SDS-PAGE technique was used to study the sera of all groups of animals. As a standard, low molecular weight protein marker was used "seven protein fractions of 66-14.2 kDa" (Fig.1). The protein profile of all animals "control and treated" were separated into 2 zones (A&B). High molecular weight proteins are placed in "Zone-A" while "Zone-B" comprises "66-14 kDa" of protein fractions having low molecular weight.

Sub-Group A1 "75 Mg/Kg Body Weight"

Protein fractions of "Zone-A" of "102, 95 and 75 kDa" of pre-treatment sera samples of sub-group A1 were constituted "8.6±0.6%, 10.4±0.45% and 12.17±0.52%" of the total serum proteins, respectively. The peaks "4, 5 and 6 of Zone B were diffused and constituted 38.17±0.6%, 16.4±0.3% and 8.23±0.8%" while peaks "7 to 9 were noticeable and constituted 18.20±1.1%, 1.50±0.3% and 4.26±0.6%" accordingly (Fig. 2a).

In Zone A, total serum proteins samples constituted "5.73±0.9%, 6.73±1.2%" and "10.2±0.3%" at 1 to 3 peaks after treatment.

4 to 6 peak levels of Zone-B became visible while peak 5 remain different. However, peak 8vanished after treatment of the samples. Except peak 4, all protein fractions indicated deterioration (Fig. 2b).

Sub-Group A2 "150 Mg/Kg Body Weight"

The protein fractions (102, 95 and 75 kDa) in Zone-A were noticeable and formed "6.56±0.4%, 9.610±1.1% and 13.6±0.5%" of total serum proteins respectively; pretreated samples of sub-group A2 (Fig. 3a). In Zone-B, except diffused peak 6, all peaks were conspicuous and peaks 4 to 9 were constituted "38.58±0.5%, 19.2±0.3%, 8.5±0.01%, 17.5±0.3%, 1.4±0.28% and 5.8±0.37%" of total serum proteins respectively (Fig. 3a). In post treated samples, the peak configuration was dissimilar. All Peaks diffused while peak 8 disappeared completely (Fig. 3b).

Sub-Group A3 "225 Mg/Kg Body Weight"

Samples of sub-group A3 showed three peaks in Zone-A, all peaks are prominent. Protein fractions of "102, 95 and 75kDa", constituted "7.4±0.3%, 6.7±0.7% and 13.9±0.7%" of the total serum proteins respectively. Peaks 4-9 in Zone B constituted "36.6±1.2%, 17.3±0.7%, 9.4±0.5%, 17.1±2.1%, 2.43±0.3% and 3.9±0.2%" respectively (Fig. 4a).

In the post-treated samples, peak "5 and 6" diffused relatively whereas peak 8 vanished totally. Almost all proteins fractions significantly ($P<0.05$) decreased while peak 4 showed (32.0%) elevation in densitometric analysis (Fig.4b)

Serum Protein Profile in Group B "Praziquantel, 10 Mg/Kg Body Weight"

Pre-treated samples of group Bshown 3 peaks in Zone-A, which represents protein fractions of "102, 95 and 75kDa", were visible and comprises "8.06±0.6%, 10.03±1.5 and 12.17±0.1%"of total serum protein respectively (Fig. 5a).

The peak configuration of diseased and cured animals, with PZQ (10 mg/kg body weight) was different. Peaks 5 and 6 showed diffusion while peak 8 totally disappeared. Peaks 2, 3 and 5 showed non-significant decline while peak 4 showed (17.0%) elevation non-significantly (Fig.5b).

Serum Protein Profile in Group F "Infected Control"

"Peak 1 (102kDa), 2 (95kDa) and 3 (75kDa)", constituted "7.5±0.3%, 8.06±0.3% and 11.87±0.5%" of total serum proteins respectively of Zone-A, group F before treatment (Fig. 6a). In Zone-B, peaks 4-9 were noticeable and constituted "38.3±0.6%, 11.10±0.3%, 9.3±0.7%, 13.2±0.5%, 2.16±0.17% and 3.2±0.8%" respectively.

At the end of experiment (on 28th day), the protein fractions of all peaks of diseased animals showed significant raise except peak 4 which showed (7.31%) decline in densitometric analysis.(Fig. 6b)".

Serum Protein Profile in Group G "Normal Control"

Samples of group G (normal animals), in Zone-A 3 peaks, demonstrating protein fraction of "102, 95 and 75 kDa" were visible and constituted "5.8±0.3%, 7.1±0.1% and 9.1±0.1%" of serum protein samples (Fig. 7a). In Zone-B, peaks 4 to 9 constituted about "48.7±1.0%, 14.17±0.6%, 3.22±0.2%, 9.26±0.25%, 0.0±0.0% and 2.73±0.1" respectively (Fig.7a).

Discussion

P. harmala (group A) showed the efficacy at dose of 75mg/kg b.w. was "16.30 & 64.2" at first and 2nd dose levels correspondingly. Whereas "150 mg/kg & 225 mg/kg bw were 65.7%, 100% and 96.6%, 100%" functioning at 1st dose and 2nd doses respectively. The analysis indicated a significant reduction in output of parasites egg at all dose levels, while in sub-groups A_2 and A_3, EPG become zero. Some scientists also described anti-parasitic activity of *Peganum harmala* against gastrointestinal tapeworms of goats. They used *Peganum harmala* seed "(100, 200 and 300 mg/kg bwat their doses level)" and their effectiveness was found "75%, 93% and 100%"correspondingly. If above mentioned dose levels were changed into doses level of presented study, "75, 150, 225 mg/kg bw" then their effectiveness (%) would be"56.2%, 69.7%, and 75% respectively". The above findings were less or more in support of the present study. The reason for "different efficacy (%) of *P. harmala* may be due to difference

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in host and parasite species". *P. harmala* and their numerous categories of extracts have been used since centuries in the ethnic medication for several diseases (Akhtar and Riffat 1986). Many researchers reported effectiveness of "Praziquantal" 100% as a safe drug against schistosomiasis ("Tianping *et al* 2006 and Raso *et al*., 2007"). Resistance risk of the "Praziquantal" cannot be ignored as describe by different researchers (Doenhof *et al*., 2008). The effect of selected herb on protein profile of animals were also examined by "SDS-PAGE" and it was noted that serum showed entire 9 peaks corresponding to 9 different protein bands, which were also studied by densitometric analysis in all diseased animals serum samples before treatment. Sera of healthy animals (control) shown 8 peaks. The peaks, 1-9 represented the protein fraction "102, 95, 75, 57, 48, 36, 24, 19 and 14 kDa, respectively".

In healthy control animals, "the peak 8 represented protein fraction (19 kDa) was found absent significantly observed in all fractions before treatment and after treatment".

After treatment, the 19 kDa protein in diseased subjects were either significantly diffused or totally vanished after treatment. It is proposed from the present study that this fraction may be antibodies or antigens. Schistosomiasis can stimulate the immune system, resulting in the antibody production. The fractions of this "nature needs a comprehensive research using western-blot analysis or proteiomics as described that 85, 37 and 20kDa protein fraction are characteristic of infections with *Schistosoma spp"* (Turrientes et *al*., 2004). Some researcher stated 27kDa protein for antibody detection against *S. japonicum* in buffaloes ("John et *al*., 2007"). The difference in fractions and results of present study may be due to difference in host species and *Schistosoma* species for immune reactivity.

Table 1: "Comparative efficacy (%) of Peganum*harmala* (Harmal, seed) with praziquantel against schistosomiasis in natural infected buffaloes"

Groups	Sub-Group (n = 9)	Treatment	Dose (mg/kg bw)	Eggs per gram of feces (EPG)					
				1st dose				2nd dose	
				0 day	3rd day	7th day	18th day	21st day	28th day
A	A1	*Peganumharmala* (Seeds)	75	800±58	700±58	667±33	667±33	600±58	300±11*
	A2		150	867±33	700±58	500±58	300±58**	100±58	0±0***
	A3		225	867±88	567±88	267±88	33±33***	0±0	0±0***
B		Praziquantel (PZQ)	10	933±33	500±58	200±58	0±0***	0±0	0±0***
C (infected)		+ve control	-	767±33	800±58	833±33	900±58	967±33	1033±00**
D (normal)		-ve control	-	0	0	0	0	0	0

"Student's t-test: * = P<0.05, ** = P<0.01, *** = P<0.001, N.S. non-significant"

Table 2:"Comparative efficacy (%) of Peganum*harmala* (Harmal, seed) with praziquantel against schistosomiasis in natural infected buffaloes"

Groups	Sub-Group (n = 9)	Treatment	Dose (mg/kg bw)	Efficacy (%)				
				1st dose			2nd dose	
				3rd day	7th day	18th day	21st day	28th day
A	A1	*Peganumharmala* (Seeds)	75	12.13±6.5	16.30±3.1	16.30±3.1	25.2±1.6	64.20±12.0b
	A2		150	19.43±4.2	42.57±5.0	65.7±5.6	88.83±6.4	100±0.0a
	A3		225	35.37±3.8	70.77±7.7	96.67±3.3	100±0.0	100±0.0
B		Praziquantel (PZQ)	10	46.63±4.6	78.83±5.4	100±0.0	100±0.0	100±0.0

ANOVA: "Comparison within group by Tukey's test: a = P<0.05, aa = P<0.01, aaa = P<0.001, comparison between same doses of different groups N.S. = non –significant, comparison of all doses with PZQ = b = P<0.05, bb = P<0.01, bbb = P<0.001"

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Table 3: "Comparison of milk production (liters), body weight (kg) and feed intake (kg)/day before and after treatment of buffaloes"

Groups	Sub-Groups	Milk production (liters) (n = 2/3)			Feed (g)/day (n = 5)			Body weight (kg) (n = 5)		
		Before treatment	After treatment	Increase↑/ decrease↓ (%)	Before treatment	After treatment	Increase↑/ decrease↓ (%)	Before treatment	After treatment	Increase↑/ decrease↓ (%)
A	A1	2.73±0.3	3.0±0.23	11.11 ↑	19±0.5	20±0.5	52.6***↑	163±4	174±7.6	6.7 ↑
	A2	2.86±0.25	5.26±0.27	85.71**↑	23.0.8	39±0.6	69±5***↑	160±4.4	174±2.7	8.7 ↑
	A3	2.53±0.12	5.4±0.26	116***↑	22.7±0.3	42.0±1.7	91.0***↑	151±4.6	167±3.3	11.9 ↑
B		2.73±0.17	6.10±0.11	126 ***↑	24.6±0.6	47±1.0	96.0***↑	160±8.7	180±1	12.5 ↑
C		3.20±0.17	1.56±0.24	53.12**↓	37±1.1	25.7±0.3	32.4 ↓	169±0.3	157±6.7	7.6 ↓
D		6.73±0.31	6.73±0.31	0.0	46.6±1.1	47.6±1.1	2.1 ↓	232±12	232±12	0.0

"Student's t-test: * = P<0.05, ** = P<0.01, *** = P<0.001, N.S. non-significant"

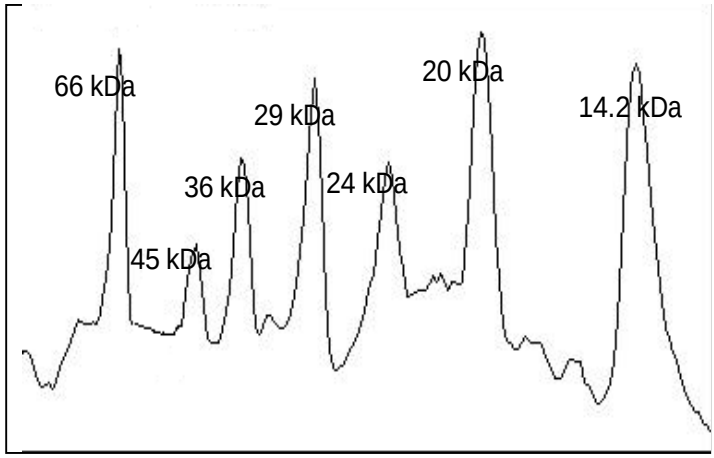

Figure 1:"Protein profile (SDS-PAGE) showing the peaks of the marker obtained by densitometric analysis (Peaks represent molecular weight in kilo Daltons (kDa)"

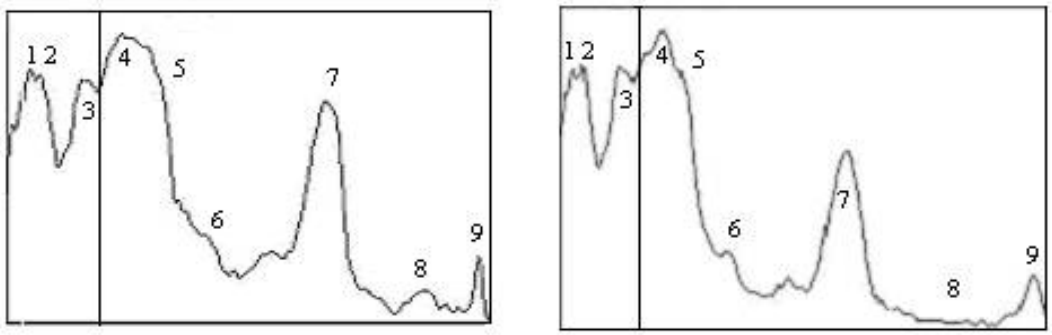

Fig 2a "before treatment" **Fig 2b"after treatment"**

Figure 2:"The densitometric analysis of infected subjects of sub-group A1 given*Peganumharmala* 75 mg/kg body weight"

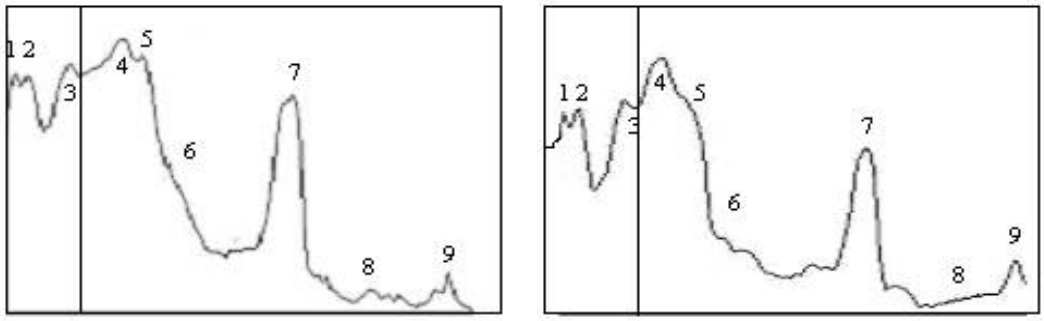

Fig 3a"before treatment" **Fig 3b"after treatment"**

Figure 3:"The densitometric analysis of infected subjects of sub-group A2 give *Peganum harmala* (150 mg/kg bw(a)before(b) after treatment"

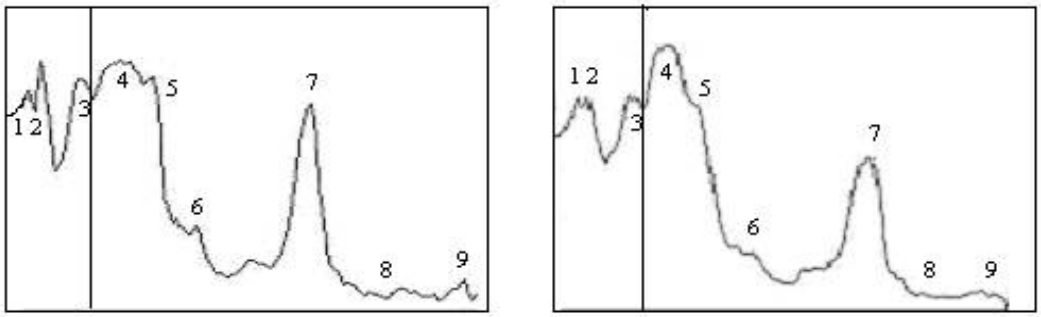

Fig 4a"before treatment" **Fig 4b"after treatment"**

Figure 4:"The densitometric analysis of infected subjects of sub-group A3 given*Peganumharmala* (225mg/kg body weight)"

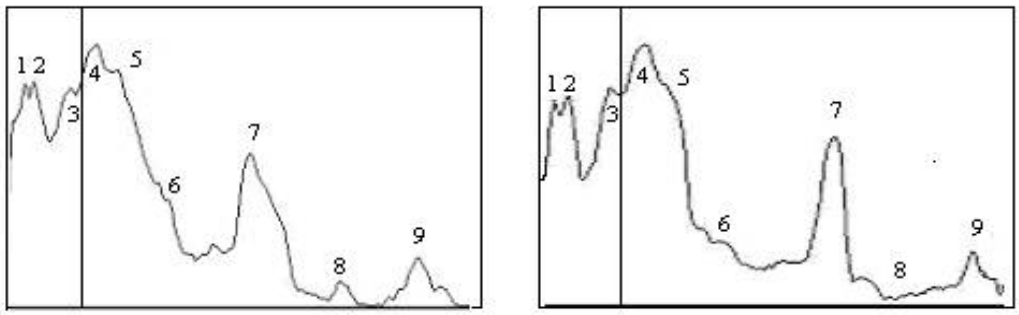

Fig 5a"before treatment" **Fig 5b"after treatment"**

Figure 5:"The densitometric analysis of infected subjects of sub-group B givenPraziquantel (10mg/kg body weight)"

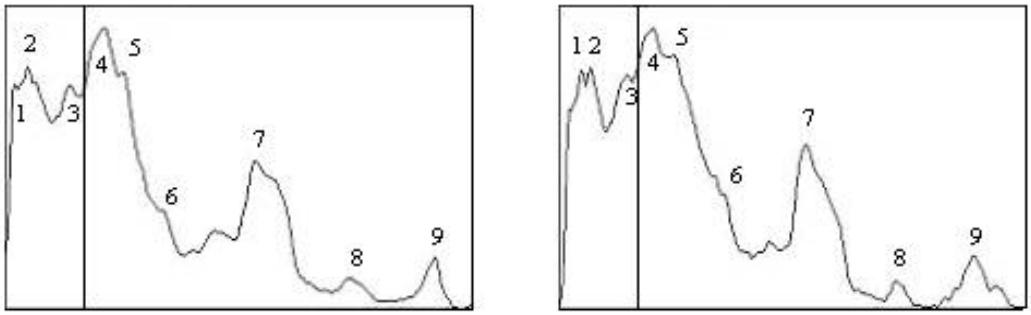

Fig 6a"before treatment" **Fig 6b"after treatment"**

Figure 6:"The densitometric analysis of infected subjects of sub-group F"

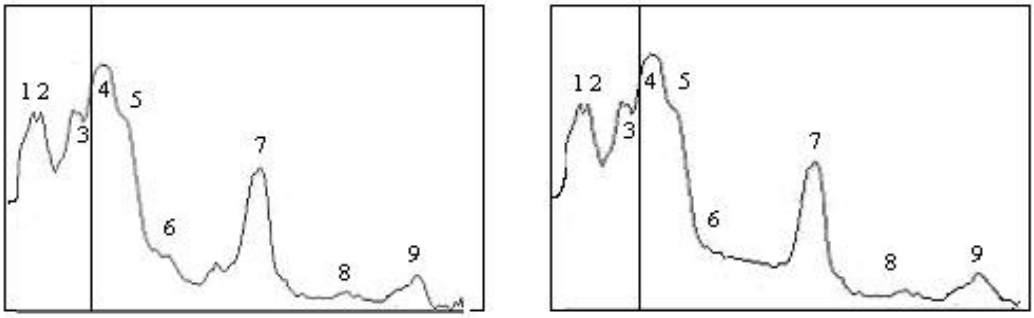

Fig 7a"before treatment" **Fig 7b"after treatment"**

Figure 7:"The densitometric analysis of healthy subjects of sub-group G"

Conclusion

From present findings, it is concluded that the herb *Peganum harmala* can easily replace Praziquantal (PZQ) with almost same efficiency (%). Furthermore, the herb is easily available at cheap price at local market and it will be good for economy of Pakistan.

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